(FILE 'HOME' ENTERED AT 12:40:51 ON 08 NOV 2002)

FILE 'BIOSIS, MEDLINE, CAPLUS, EMBASE' ENTERED AT 12:41:01 ON 08 NOV 2002

L1	16	ACYL COA CHOLESTEROL ACYLTRANSFERASE 2
L2	3041	ACYL COA CHOLESTEROL ACYLTRANSFERASE
L3	14525135	2
L4	1666	L2 AND L3
L5	3839	ACAT
L6	2145	L5 AND L3
L7	76703	ANTISENS?
L8	15069	RIBOZYM?
L9	0	L1 AND L7
L10	0	L1 AND L8
L11	10	L2 AND L7
L12	7	DUP REM L11 (3 DUPLICATES REMOVED)
L13	0	L2 AND L8
L14	19	L5 AND L7
L15	8	DUP REM L14 (11 DUPLICATES REMOVED)
L16	0	L2 AND L8
L17	3	L5 AND L8
L18	1	DUP REM L17 (2 DUPLICATES REMOVED)

L12 ANSWER 1 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 1998:88114 BIOSIS
DOCUMENT NUMBER: PREV199800088114
TITLE: Effect of reduced low-density lipoprotein receptor level on HepG2 cell cholesterol metabolism.
AUTHOR(S): Izem, Lahoucine; Rassart, Eric; Kamate, Lassana; Falstrault, Louise; Rhainds, David; Brissette, Louise
CORPORATE SOURCE: Dep. Sci. Biol., Univ. Quebec Montreal, C.P. 8888, Succ. Centre-ville, Montreal, PQ H3C 3P8 Canada
SOURCE: Biochemical Journal, (Jan. 1, 1998) Vol. 329, No. 1, pp. 81-88.
ISSN: 0264-6021.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Low-density lipoproteins (LDL) are taken up by both LDL receptor (LDLr)-dependent and -independent pathways. In order to determine the importance of these pathways in the activity of the various enzymes that are important in maintaining the cellular cholesterol level in hepatic cells, we created HepG2 cells expressing lower levels of LDLr. Thus HepG2 cells were transfected with a constitutive expression vector (pRc/CMV) containing a fragment of LDLr cDNA inserted in an **antisense** manner. Stable transformants were obtained that showed significant reductions of 42, 72 and 85% of LDLr protein levels compared with the control, as demonstrated by immunoblotting and confirmed by the LDL binding assay. The best inactivation was achieved with the construct containing the first 0.7 kb of LDLr cDNA. Incubating the different HepG2 cell subtypes with LDL showed similar association of apolipoprotein B (apo B) or cholesteryl esters from LDL with the cells, indicating that the LDLr deficiency did not significantly affect LDL uptake by the cell. However, apoB degradation was reduced significantly by 71-82% in the most LDLr-deficient HepG2 cells. We also found that 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCoA red) activity is significantly increased by 32-35% in HepG2 cells expressing very low levels of LDLr that also demonstrate a significant decrease of 20% in **acyl-CoA**: **cholesterol acyltransferase** (ACAT) activity. However, these effects are moderate compared with those observed when cells were incubated in lipoprotein-depleted medium, where a > 900% increase in HMGCoA red activity and a loss of 60 % of ACAT activity was observed. Thus, in HepG2 cells, different levels of LDLr affect LDL-apoB degradation, but have very little effect on LDL association, HMGCoA red and ACAT activities, revealing that LDLr is more important in the clearance of LDL-apoB than in HepG2 cell cholesterol homeostasis, a role that should be attributable to both LDLr-dependent and -independent pathways.

L12 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:168132 CAPLUS
DOCUMENT NUMBER: 134:218021
TITLE: Nucleic acids encoding plant sterol acyltransferases and their use to modify sterol composition
INVENTOR(S): Lassner, Michael; Van Eenennaam, Alison
PATENT ASSIGNEE(S): Monsanto Company, USA
SOURCE: PCT Int. Appl., 127 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001016308	A2	20010308	WO 2000-US23863	20000830
WO 2001016308	A3	20020117		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,

CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

BR 2000014154	A	20020507	BR 2000-14154	20000830
EP 1210417	A2	20020605	EP 2000-959644	20000830

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL

PRIORITY APPLN. INFO.:	US 1999-152493P	P	19990830
	WO 2000-US23863	W	20000830

AB The present invention is directed to lecithin:cholesterol acyltransferase-like polypeptides (LCAT) and **acyl CoA: cholesterol acyltransferases**-like polypeptides (ACAT). The invention provides polynucleotides encoding such cholesterol:acyltransferase-like polypeptides, polypeptides encoded by such polynucleotides, and the use of such polynucleotides to alter sterol compn. and oil prodn. in plants and host cells. Four LCAT cDNAs are provided from Arabidopsis thaliana, as well as 2 genomic DNAs encoding LCAT from A. thaliana, 7 ESTs from soybean and 11 ESTs from corn. ACAT-encoding ESTs are also identified from A. thaliana, soybean, maize, and Mortierella alpina. Also provided are oils produced by the plants and host cells contg. the polynucleotides and food products, nutritional supplements, and pharmaceutical compn. contg. plants or oils of the present invention.

L12 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER:	1999:784271 CAPLUS
DOCUMENT NUMBER:	132:31788
TITLE:	plant and animal and fungal **Acyl coa :cholesterol acyltransferase** related nucleic acid sequences with utility in altering lipid composition of plant oils
INVENTOR(S):	Lassner, Michael W.; Ruezinsky, Diane M.
PATENT ASSIGNEE(S):	Calgene LLC, USA
SOURCE:	PCT Int. Appl., 89 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9963096	A2	19991209	WO 1999-US12541	19990604
WO 9963096	A3	20000127		

W: CA, JP, MX, US

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

CA 2330180	AA	19991209	CA 1999-2330180	19990604
EP 1084256	A2	20010321	EP 1999-955296	19990604

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

JP 2002517201	T2	20020618	JP 2000-552290	19990604
US 6444876	B1	20020903	US 1999-326203	19990604

PRIORITY APPLN. INFO.:	US 1998-88143P	P	19980605
	US 1998-108389P	P	19981112
	WO 1999-US12541	W	19990604

AB By this invention, novel nucleic acid sequences encoding for Arabidopsis thaliana or soybean or corn or Brassica or safflower or alfalfa or sunflower **acyl-CoA:cholesterol**

acyltransferase (ACAT) related proteins are provided, wherein said ACAT-like protein is active in the formation of a sterol ester and/or triacylglycerol from a fatty acyl-CoA and sterol and/or diacylglycerol substrates. Non-plant sources include rat, human, mouse, Mortierella, or Caenorhabditis elegans. Also considered are amino acid and nucleic acid sequences obtainable from ACAT-like nucleic acid sequences and the use of such sequences to provide transgenic host cells capable of producing sterol esters and/or triacylglycerols (lipid compn.). Baculovirus expression systems are described. Transgenic Brassica plants are described also. Expression suppression of this protein is achieved using **antisense** RNA.

L12 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:503852 CAPLUS
DOCUMENT NUMBER: 131:255269
TITLE: Selective uptake of cholesteryl ester from low density lipoprotein is involved in HepG2 cell cholesterol homeostasis
AUTHOR(S): Charest, Marie-Claude; Rhainds, David; Falstrault, Louise; Matzouranis, Tony; Brissette, Louise
CORPORATE SOURCE: Departement des Sciences Biologiques, Universite du Quebec a Montreal, Montreal, QC, H3C 3P8, Can.
SOURCE: European Journal of Biochemistry (1999), 263(2), 402-409
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Low d. lipoprotein (LDL) can follow either a holoparticle uptake pathway, initiated by the LDL receptor (LDLr), and be completely degraded, or it can deliver its cholesteryl esters (CE) selectively to HepG2 cells. Although high d. lipoprotein-CE selective uptake has been shown to be linked to cell cholesterol homeostasis in nonhepatic cells, there is no available information on the effect of LDL-CE selective uptake on hepatic cell cholesterol homeostasis. In order to define the role of the LDL-CE selective uptake pathway in hepatic cell cholesterol homeostasis, we used a cellular model that expresses constitutively a LDLr **antisense** mRNA and that shows LDLr activity at 31% the normal level (HepG2-all cells). The addn. of a specific antibody anti-LDLr (IgG-C7) reduces LDL protein degrdn. (LDLr activity) to 7%. This cellular model therefore reflects, above all, LDL-CE selective uptake activity when incubated with LDL. The inactivation of LDLr reduces LDL-protein assocn. by 78% and LDL-CE assocn. by only 43%. The LDL-CE selective uptake was not reduced by the inactivation of LDLr. The activities of the various enzymes involved in cell cholesterol homeostasis were measured in normal and LDLr-deficient cells during incubation in the absence or presence of LDL as a cholesterol source. Essentially, 3-hydroxy-3-methylglutaryl CoA reductase and **acyl CoA:cholesterol acyltransferase** (ACAT) activities responded to LDL in LDLr-deficient cells as well as in normal HepG2 cells. Inhibition of lysosomal hydrolysis with chloroquine abolished the effect measured on ACAT activity in the presence of LDL, suggesting that CE of LDL, but not free cholesterol, maintains cell cholesterol homeostasis. Thus, in HepG2 cells, when LDLr function is virtually abolished, LDL-CE selective uptake is coupled to cell cholesterol homeostasis.
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:805749 CAPLUS
DOCUMENT NUMBER: 128:72367
TITLE: DNA encoding human acyl-coenzyme A:cholesterol acyltransferases II and III and their therapeutic uses
INVENTOR(S): Sturley, Stephen L.

PATENT ASSIGNEE(S): Trustees of Columbia University In the City of New York, USA; Sturley, Stephen L.
SOURCE: PCT Int. Appl., 120 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9745439	A1	19971204	WO 1997-US9460	19970530
W: AU, CA, JP, MX, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9732259	A1	19980105	AU 1997-32259	19970530
PRIORITY APPLN. INFO.:			US 1996-657620	19960530
			WO 1997-US9460	19970530

AB This invention provides isolated cDNAs encodes human **acyl-CoA:cholesterol acyltransferases** II or III. To use yeast genetics to study sterol esterification, human **acyl-CoA:cholesterol acyltransferase** I (ACAT-I) was used to search for homologous yeast genes and subsequently to identify an addnl. human isoform. Two yeast genes (ARE1 and ARE2) were isolated and the roles of their enzyme products in sterol esterification were characterized. PCR-based strategies were used to isolate and characterize full-length cDNA clones of human ACAT-II and ACAT-III (ARGP1 and ARGP2, resp.). Tissue-specific expression of the human clones suggests that ARGP1 is an ideal candidate for sterol esterification in tissues such as liver and intestine, whereas ARGP2 is an embryonic isoform of the ACAT gene family. This invention also provides various methods for inhibiting wild-type **acyl-CoA:cholesterol acyltransferase** II or III in a subject. This invention also provides a method for identifying a chem. compd. which is capable of inhibiting **acyl-CoA:cholesterol acyltransferase** II or III and a pharmaceutical compn. comprising of the chem. compd. identified by the above-described method. This invention also provides a method of treating a subject who has atherosclerosis or hyperlipidemia.

L12 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:475329 CAPLUS
DOCUMENT NUMBER: 121:75329
TITLE: Molecular cloning of cDNA for human acyl coenzyme A: cholesterol acyltransferase
INVENTOR(S): Chang, Ta-Ywan; Chang, Catherine C. Y.
PATENT ASSIGNEE(S): Dartmouth College, USA
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9409126	A2	19940428	WO 1993-US9704	19931012
WO 9409126	A3	19940623		
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5484727	A	19960116	US 1993-121057	19930910
AU 9453566	A1	19940509	AU 1994-53566	19931012
PRIORITY APPLN. INFO.:			US 1992-959950	19921014
			US 1993-121057	19930910
			WO 1993-US9704	19931012

AB A 4.1-kb cDNA fragment encoding biol. active **acyl CoA: cholesterol acyltransferase** (ACAT) was cloned from human macrophage cell line THP-1 cells. The nucleic acid, or a fragment thereof, may be ligated with an expression vector and transfected into cells to express **acyl CoA: cholesterol acyltransferase** activity in intact cells and in cell-free exts. It can be used for the prepn. of **antisense** oligonucleotides for reducing the cholesterol level in transgenic animals.

L12 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:93342 CAPLUS
DOCUMENT NUMBER: 123:77917
TITLE: Purification, cloning, and expression of a human enzyme with acyl coenzyme A:Cholesterol acyltransferase activity, which is identical to liver carboxylesterase
AUTHOR(S): Becker, Alfred; Boettcher, Alfred; Lackner, Karl J.; Fehringer, Petra; Notka, Frank; Aslanidis, Charalampos; Schmitz, Gerd
CORPORATE SOURCE: Institute Clinical Chemistry and Laboratory Medicine, University Regensburg, Regensburg, 93042, Germany
SOURCE: Arteriosclerosis and Thrombosis (1994), 14(8), 1346-55
CODEN: ARTTE5; ISSN: 1049-8834
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An enzyme with **acyl CoA:cholesterol acyltransferase** (ACAT) activity was isolated from porcine liver, and sequences derived from trypsinized peptides indicated homol. to liver carboxylesterase. By use of degenerate primers, human cDNA clones were identified, which were identical to human liver carboxylesterase. Expression of the full-length cDNA in Chinese hamster ovary (CHO) cells led to an approx. threefold increase in cellular ACAT activity. This was accompanied by an .apprx.20-fold increase of cellular cholesteryl ester content. By light and electron microscopy, recombinant CHO cells contained numerous lipid droplets that were not present in control CHO cells. Expression of an **antisense** cDNA in HepG2 cells reduced cellular ACAT activity by 35% compared with control. To further investigate the role of the enzyme in cellular cholesterol homeostasis, regulation of the mRNA was investigated in 7-day cultured human mononuclear phagocytes (MNPs). When these cells were incubated in lipoprotein-deficient serum for 18 h, the mRNA for ACAT/carboxylesterase was almost not detectable on Northern blots, whereas after incubation with acetylated low-d. lipoproteins, a strong hybridization signal was obtained. This is evidence that the mRNA of ACAT/carboxylesterase is induced by cholesterol loading. It is concluded from the data presented that ACAT/carboxylesterase is relevant for cellular cholesterol esterification in vivo. The regulation in MNPs indicates that the enzyme is also involved in foam cell formation during early atherogenesis.

L15 ANSWER 1 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 1999:414537 BIOSIS
DOCUMENT NUMBER: PREV199900414537
TITLE: Selective uptake of cholesteryl ester from low density lipoprotein is involved in HepG2 cell cholesterol homeostasis.
AUTHOR(S): Charest, Marie-Claude; Rhainds, David; Falstrault, Louise; Matzouranis, Tony; Brissette, Louise (1)
CORPORATE SOURCE: (1) Departement des Sciences Biologiques, Universite du Quebec a Montreal, Succ. Centre-Ville, Montreal, PQ, H3C 3P8 Canada
SOURCE: European Journal of Biochemistry, (July, 1999) Vol. 263, No. 2, pp. 402-409.
ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Low density lipoprotein (LDL) can follow either a holoparticle uptake pathway, initiated by the LDL receptor (LDLr), and be completely degraded, or it can deliver its cholesteryl esters (CE) selectively to HepG2 cells. Although high density lipoprotein-CE selective uptake has been shown to be linked to cell cholesterol homeostasis in nonhepatic cells, there is no available information on the effect of LDL-CE selective uptake on hepatic cell cholesterol homeostasis. In order to define the role of the LDL-CE selective uptake pathway in hepatic cell cholesterol homeostasis, we used a cellular model that expresses constitutively a LDLr **antisense** mRNA and that shows LDLr activity at 31% the normal level (HepG2-all cells). The addition of a specific antibody anti-LDLr (IgG-C7) reduces LDL protein degradation (LDLr activity) to 7%. This cellular model therefore reflects, above all, LDL-CE selective uptake activity when incubated with LDL. The inactivation of LDLr reduces LDL-protein association by 78% and LDL-CE association by only 43%. The LDL-CE selective uptake was not reduced by the inactivation of LDLr. The activities of the various enzymes involved in cell cholesterol homeostasis were measured in normal and LDLr-deficient cells during incubation in the absence or presence of LDL as a cholesterol source. Essentially, 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A:cholesterol acyltransferase (**ACAT**) activities responded to LDL in LDLr-deficient cells as well as in normal HepG2 cells. Inhibition of lysosomal hydrolysis with chloroquine abolished the effect measured on **ACAT** activity in the presence of LDL, suggesting that CE of LDL, but not free cholesterol, maintains cell cholesterol homeostasis. Thus, in HepG2 cells, when LDLr function is virtually abolished, LDL-CE selective uptake is coupled to cell cholesterol homeostasis.

L15 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
ACCESSION NUMBER: 1998:88114 BIOSIS
DOCUMENT NUMBER: PREV199800088114
TITLE: Effect of reduced low-density lipoprotein receptor level on HepG2 cell cholesterol metabolism.
AUTHOR(S): Izem, Lahoucine; Rassart, Eric; Kamate, Lassana; Falstrault, Louise; Rhainds, David; Brissette, Louise
CORPORATE SOURCE: Dep. Sci. Biol., Univ. Quebec Montreal, C.P. 8888, Succ. Centre-ville, Montreal, PQ H3C 3P8 Canada
SOURCE: Biochemical Journal, (Jan. 1, 1998) Vol. 329, No. 1, pp. 81-88.
ISSN: 0264-6021.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Low-density lipoproteins (LDL) are taken up by both LDL receptor (LDLr)-dependent and -independent pathways. In order to determine the importance of these pathways in the activity of the various enzymes that are important in maintaining the cellular cholesterol level in hepatic cells, we created HepG2 cells expressing lower levels of LDLr. Thus HepG2

cells were transfected with a constitutive expression vector (pRc/CMV) containing a fragment of LDLr cDNA inserted in an **antisense** manner. Stable transformants were obtained that showed significant reductions of 42, 72 and 85% of LDLr protein levels compared with the control, as demonstrated by immunoblotting and confirmed by the LDL binding assay. The best inactivation was achieved with the construct containing the first 0.7 kb of LDLr cDNA. Incubating the different HepG2 cell subtypes with LDL showed similar association of apolipoprotein B (apo B) or cholesteryl esters from LDL with the cells, indicating that the LDLr deficiency did not significantly affect LDL uptake by the cell. However, apoB degradation was reduced significantly by 71-82% in the most LDLr-deficient HepG2 cells. We also found that 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCoA red) activity is significantly increased by 32-35% in HepG2 cells expressing very low levels of LDLr that also demonstrate a significant decrease of 20% in acyl-CoA: cholesterol acyltransferase (**ACAT**) activity. However, these effects are moderate compared with those observed when cells were incubated in lipoprotein-depleted medium, where a > 900% increase in HMGCoA red activity and a loss of 60 % of **ACAT** activity was observed. Thus, in HepG2 cells, different levels of LDLr affect LDL-apoB degradation, but have very little effect on LDL association, HMGCoA red and **ACAT** activities, revealing that LDLr is more important in the clearance of LDL-apoB than in HepG2 cell cholesterol homeostasis, a role that should be attributable to both LDLr-dependent and -independent pathways.

L15 ANSWER 3 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
ACCESSION NUMBER: 1998:135263 BIOSIS
DOCUMENT NUMBER: PREV199800135263
TITLE: Regulation of CAT protein by ribozyme and **antisense** mRNA in transgenic mice.
AUTHOR(S): Sokol, Deborah L.; Passey, Robert J.; Mackinlay, Anthony G.; Murray, James D. (1)
CORPORATE SOURCE: (1) Dep. Anim. Sci., Univ. Calif., Davis, CA 95616 USA
SOURCE: Transgenic Research, (Jan., 1998) Vol. 7, No. 1, pp. 41-50. ISSN: 0962-8819.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Transgenic mouse lines were engineered to express stably **antisense** mRNA or **antisense** mRNA containing catalytic ribozyme (rbz) structures complementary to bacterial chloramphenicol acetyltransferase (CAT) gene transcripts. One transgenic line expressed **antisense** mRNA that specifically targeted full-length CAT coding sequences (**ACAT**). Another transgenic line expressed full-length **antisense** CAT mRNA which was modified by mutagenesis to include four rbz cassettes (rbz-**ACAT**) in order to compare **antisense** versus **antisense**-rbz function in vivo. Preliminary data were also collected from a transgenic mouse line expressing **antisense** mRNA targeting 72% of the 5' region of CAT coding sequences (5' **ACAT**). All constructs contained similar control elements in their design. Promoter elements were derived from the bovine alphas1-casein gene, while the small t intron and 3' control sequences were derived from SV40. The ability of these various constructs to down-regulate CAT protein levels was compared by analysis of CAT protein production in lactating double-hemizygous transgenic female mice. Every double-hemizygous mouse analysed expressed mRNA from the alphas1-casein-CAT construct (Clarke et al., 1994) and equivalent levels of mRNA from one of the three **antisense** constructs. Transgenic mouse lines expressing both **ACAT** and CAT mRNA down-regulated CAT protein levels by 90% of that found in the CAT only transgenic population. Similarly, double-hemizygous transgenic lines expressing both rbz-**ACAT** and CAT mRNA regulated CAT protein levels by 87%. Preliminary data suggests that expression of mRNA from 5' **ACAT**/CAT double-hemizygote mice allowed approximately 67% down-regulation of normal CAT protein levels. We conclude that incorporation of multiple ribozymes

within the full-length **antisense** CAT construct does not enhance the effectiveness of **antisense** mRNA in the down-regulation of CAT protein production in our system.

L15 ANSWER 4 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
ACCESSION NUMBER: 1994:445646 BIOSIS
DOCUMENT NUMBER: PREV199497458646
TITLE: Purification, cloning and expression of a human enzyme with acyl coenzyme A: Cholesterol acyltransferase activity, which is identical to liver carboxylesterase.
AUTHOR(S): Becker, Alfred; Boettcher, Alfred; Lackner, Karl J.; Fehringer, Petra; Notka, Frank; Aslanidis, Charalampos; Schmitz, Gerd (1)
CORPORATE SOURCE: (1) Institut Klinische Chemie Laboratoriumsmedizin, Klinikum Universitaet Regensburg, 93042 Regensburg Germany
SOURCE: Arteriosclerosis and Thrombosis, (1994) Vol. 14, No. 8, pp. 1346-1355.
ISSN: 1049-8834.
DOCUMENT TYPE: Article
LANGUAGE: English
AB An enzyme with acyl coenzyme A:cholesterol acyltransferase (**ACAT**) activity was isolated from porcine liver, and sequences derived from trypsinized peptides indicated homology to liver carboxylesterase. By use of degenerate primers, human cDNA clones were identified, which were identical to human liver carboxylesterase. Expression of the full-length cDNA in Chinese hamster ovary (CHO) cells led to an approximately threefold increase in cellular **ACAT** activity. This was accompanied by an apprxeq 20-fold increase of cellular cholesteryl ester content. By light and electron microscopy, recombinant CHO cells contained numerous lipid droplets that were not present in control CHO cells. Expression of an **antisense** cDNA in HepG2 cells reduced cellular **ACAT** activity by 35% compared with control. To further investigate the role of the enzyme in cellular cholesterol homeostasis, regulation of the mRNA was investigated in 7-day cultured human mononuclear phagocytes (MNPs). When these cells were incubated in lipoprotein-deficient serum for 18 hours, the mRNA for **ACAT**/carboxylesterase was almost not detectable on Northern blots, whereas after incubation with acetylated low-density lipoproteins, a strong hybridization signal was obtained. This is evidence that the mRNA of **ACAT**/carboxylesterase is induced by cholesterol loading. It is concluded from the data presented that **ACAT**/carboxylesterase is relevant for cellular cholesterol esterification in vivo. The regulation in MNPs indicates that the enzyme is also involved in foam cell formation during early atherogenesis.

L15 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:168132 CAPLUS
DOCUMENT NUMBER: 134:218021
TITLE: Nucleic acids encoding plant sterol acyltransferases and their use to modify sterol composition
INVENTOR(S): Lassner, Michael; Van Eenennaam, Alison
PATENT ASSIGNEE(S): Monsanto Company, USA
SOURCE: PCT Int. Appl., 127 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001016308	A2	20010308	WO 2000-US23863	20000830
WO 2001016308	A3	20020117		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,

HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

BR 2000014154	A	20020507	BR 2000-14154	20000830
EP 1210417	A2	20020605	EP 2000-959644	20000830

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL

PRIORITY APPLN. INFO.: US 1999-152493P P 19990830
WO 2000-US23863 W 20000830

AB The present invention is directed to lecithin:cholesterol acyltransferase-like polypeptides (LCAT) and acyl CoA:cholesterol acyltransferases-like polypeptides (**ACAT**). The invention provides polynucleotides encoding such cholesterol:acyltransferase-like polypeptides, polypeptides encoded by such polynucleotides, and the use of such polynucleotides to alter sterol compn. and oil prodn. in plants and host cells. Four LCAT cDNAs are provided from Arabidopsis thaliana, as well as 2 genomic DNAs encoding LCAT from A. thaliana, 7 ESTs from soybean and 11 ESTs from corn. **ACAT**-encoding ESTs are also identified from A. thaliana, soybean, maize, and Mortierella alpina. Also provided are oils produced by the plants and host cells contg. the polynucleotides and food products, nutritional supplements, and pharmaceutical compn. contg. plants or oils of the present invention.

L15 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:784271 CAPLUS
DOCUMENT NUMBER: 132:31788
TITLE: plant and animal and fungal Acyl coa:cholesterol acyltransferase related nucleic acid sequences with utility in altering lipid composition of plant oils
INVENTOR(S): Lassner, Michael W.; Ruezinsky, Diane M.
PATENT ASSIGNEE(S): Calgene LLC, USA
SOURCE: PCT Int. Appl., 89 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9963096	A2	19991209	WO 1999-US12541	19990604
WO 9963096	A3	20000127		

W: CA, JP, MX, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

CA 2330180	AA	19991209	CA 1999-2330180	19990604
EP 1084256	A2	20010321	EP 1999-955296	19990604

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

JP 2002517201	T2	20020618	JP 2000-552290	19990604
US 6444876	B1	20020903	US 1999-326203	19990604

PRIORITY APPLN. INFO.: US 1998-88143P P 19980605
US 1998-108389P P 19981112
WO 1999-US12541 W 19990604

AB By this invention, novel nucleic acid sequences encoding for Arabidopsis thaliana or soybean or corn or Brassica or safflower or alfalfa or sunflower acyl-CoA:cholesterol acyltransferase (**ACAT**) related proteins are provided, wherein said **ACAT**-like protein is active in the formation of a sterol ester and/or triacylglycerol from a fatty acyl-CoA and sterol and/or diacylglycerol substrates. Non-plant sources

include rat, human, mouse, Mortierella, or Caenorhabditis elegans. Also considered are amino acid and nucleic acid sequences obtainable from **ACAT**-like nucleic acid sequences and the use of such sequences to provide transgenic host cells capable of producing sterol esters and/or triacylglycerols (lipid compn.). Baculovirus expression systems are described. Transgenic Brassica plants are described also. Expression suppression of this protein is achieved using **antisense** RNA.

L15 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:805749 CAPLUS
DOCUMENT NUMBER: 128:72367
TITLE: DNA encoding human acyl-coenzyme A:cholesterol acyltransferases II and III and their therapeutic uses
INVENTOR(S): Sturley, Stephen L.
PATENT ASSIGNEE(S): Trustees of Columbia University In the City of New York, USA; Sturley, Stephen L.
SOURCE: PCT Int. Appl., 120 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9745439	A1	19971204	WO 1997-US9460	19970530
W: AU, CA, JP, MX, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9732259	A1	19980105	AU 1997-32259	19970530

PRIORITY APPLN. INFO.: US 1996-657620 19960530
WO 1997-US9460 19970530

AB This invention provides isolated cDNAs encodes human acyl-CoA:cholesterol acyltransferases II or III. To use yeast genetics to study sterol esterification, human acyl-CoA:cholesterol acyltransferase I (**ACAT**-I) was used to search for homologous yeast genes and subsequently to identify an addnl. human isoform. Two yeast genes (ARE1 and ARE2) were isolated and the roles of their enzyme products in sterol esterification were characterized. PCR-based strategies were used to isolate and characterize full-length cDNA clones of human **ACAT**-II and **ACAT**-III (ARGP1 and ARGP2, resp.). Tissue-specific expression of the human clones suggests that ARGP1 is an ideal candidate for sterol esterification in tissues such as liver and intestine, whereas ARGP2 is an embryonic isoform of the **ACAT** gene family. This invention also provides various methods for inhibiting wild-type acyl-CoA:cholesterol acyltransferase II or III in a subject. This invention also provides a method for identifying a chem. compd. which is capable of inhibiting acyl-CoA:cholesterol acyltransferase II or III and a pharmaceutical compn. comprising of the chem. compd. identified by the above-described method. This invention also provides a method of treating a subject who has atherosclerosis or hyperlipidemia.

L15 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:475329 CAPLUS
DOCUMENT NUMBER: 121:75329
TITLE: Molecular cloning of cDNA for human acyl coenzyme A: cholesterol acyltransferase
INVENTOR(S): Chang, Ta-Ywan; Chang, Catherine C. Y.
PATENT ASSIGNEE(S): Dartmouth College, USA
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9409126	A2	19940428	WO 1993-US9704	19931012
WO 9409126	A3	19940623		

W: AU, CA, JP

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

US 5484727	A	19960116	US 1993-121057	19930910
AU 9453566	A1	19940509	AU 1994-53566	19931012

PRIORITY APPLN. INFO.:

US 1992-959950	19921014
US 1993-121057	19930910
WO 1993-US9704	19931012

AB A 4.1-kb cDNA fragment encoding biol. active acyl CoA:cholesterol acyltransferase (**ACAT**) was cloned from human macrophage cell line THP-1 cells. The nucleic acid, or a fragment thereof, may be ligated with an expression vector and transfected into cells to express acyl CoA: cholesterol acyltransferase activity in intact cells and in cell-free exts. It can be used for the prepn. of **antisense** oligonucleotides for reducing the cholesterol level in transgenic animals.

L18 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 1998:135263 BIOSIS
DOCUMENT NUMBER: PREV199800135263
TITLE: Regulation of CAT protein by **ribozyme** and antisense mRNA in transgenic mice.
AUTHOR(S): Sokol, Deborah L.; Passey, Robert J.; Mackinlay, Anthony G.; Murray, James D. (1)
CORPORATE SOURCE: (1) Dep. Anim. Sci., Univ. Calif., Davis, CA 95616 USA
SOURCE: Transgenic Research, (Jan., 1998) Vol. 7, No. 1, pp. 41-50. ISSN: 0962-8819.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Transgenic mouse lines were engineered to express stably antisense mRNA or antisense mRNA containing catalytic **ribozyme** (rbz) structures complementary to bacterial chloramphenicol acetyltransferase (CAT) gene transcripts. One transgenic line expressed antisense mRNA that specifically targeted full-length CAT coding sequences (**ACAT**). Another transgenic line expressed full-length antisense CAT mRNA which was modified by mutagenesis to include four rbz cassettes (rbz-**ACAT**) in order to compare antisense versus antisense-rbz function in vivo. Preliminary data were also collected from a transgenic mouse line expressing antisense mRNA targeting 72% of the 5' region of CAT coding sequences (5' **ACAT**). All constructs contained similar control elements in their design. Promoter elements were derived from the bovine alphas1-casein gene, while the small t intron and 3' control sequences were derived from SV40. The ability of these various constructs to down-regulate CAT protein levels was compared by analysis of CAT protein production in lactating double-hemizygous transgenic female mice. Every double-hemizygous mouse analysed expressed mRNA from the alphas1-casein-CAT construct (Clarke et al., 1994) and equivalent levels of mRNA from one of the three antisense constructs. Transgenic mouse lines expressing both **ACAT** and CAT mRNA down-regulated CAT protein levels by 90% of that found in the CAT only transgenic population. Similarly, double-hemizygous transgenic lines expressing both rbz-**ACAT** and CAT mRNA regulated CAT protein levels by 87%. Preliminary data suggests that expression of mRNA from 5' **ACAT**/CAT double-hemizygote mice allowed approximately 67% down-regulation of normal CAT protein levels. We conclude that incorporation of multiple **ribozymes** within the full-length antisense CAT construct does not enhance the effectiveness of antisense mRNA in the down-regulation of CAT protein production in our system.

=>